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Is 20S RNA Naked?

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The 20S RNA of *Saccharomyces cerevisiae* is a single-stranded, circular RNA virus. A previous study suggested that this RNA is part of a 32S ribonucleoprotein particle, being associated with multiple copies of a 23-kilodalton protein. We show here that this protein is, in fact, the chromosome-encoded heat shock protein Hsp26. Furthermore, it is apparently not associated with 20S RNA and plays no obvious role in the life cycle of the virus.

When logarithmically growing cells of *Saccharomyces cerevisiae* are shifted from a nutritionally rich medium to a poor medium, such as 1% potassium acetate, the cells sporulate and an RNA species, estimated by gel electrophoresis to be 20S, accumulates to very high levels (5, 6). Originally, this RNA was thought to be an rRNA precursor, but it was subsequently shown to be a cytoplasmically inherited trait completely unrelated to sporulation (4). Furthermore, the RNA appeared to be associated with 18 to 20 copies of a 23-kilodalton (kDa) polypeptide in a 32S particle (18). Matsumoto et al. have extended these studies and have shown that the 20S RNA is a circular, single-stranded RNA virus which replicates via an RNA-to-RNA pathway (10).

Susek and Lindquist (15) recently made the observation that the *S. cerevisiae* heat-shock protein gene *HSP26* encodes a polypeptide with a predicted size (27 kDa) and amino acid composition (3, 15) very similar to that determined for the 23-kDa protein associated with the 20S RNA (18) and that these proteins may thus be identical. Also, upon heat shock, several species, including *S. cerevisiae*, form aggregates which contain 20 copies of the Hsp26 protein and which sediment as 20S particles in sucrose gradients (1, 2, 8, 14, 17). There have been reports of an association of these particles with cellular mRNA as well (12). The sequence of over 90% of the 20S RNA genome shows no open reading frame or part thereof whose encoded protein has a composition consistent with that of the 23-kDa protein (11). Finally, a connection between 20S RNA and Hsp26 is consistent with the fact that the synthesis of both molecules is induced under stress conditions, such as heat shock and starvation.

In order to determine whether the 23-kDa protein which is associated with the 20S RNA is in fact Hsp26, we examined particles obtained from the three strains listed in Table 1. These strains were grown at 30°C in 50 ml of YPAD broth (1% yeast extract, 2% peptone, 2% dextrose, 0.04% adenine sulfate) to late log-early stationary phase. The cells were washed with 10 ml of sterile distilled water, pelleted, and transferred to 1 liter of 1% potassium acetate to induce 20S RNA production. After an 18-h induction with vigorous shaking at 30°C, spheroplasts were prepared using Mureinase (U.S. Biochemicals), gently pelleted, resuspended in 5 ml of ice-cold extraction buffer (18) (containing 10 mM Tris, pH 7.4, 30 mM $MgCl_2$, 100 mM NaCl, heparin at 200 μg/ml, and the protease inhibitor phenylmethylsulfonyl fluoride at a concentration of 1 mM), and gently broken by using a ceramic tissue homogenizer. Lysates were clarified (10 min at 10,000 × *g*) and applied to 5 to 20% linear sucrose gradients as described by Wejksnora and Haber (18). Twenty-two 1.7-ml fractions were pumped from each gradient.

Nucleic acids were purified from 500 μl of each fraction by phenol extraction followed by ethanol precipitation. Dried pellets were resuspended in 30 μl of standard loading buffer (9) containing bentonite at 5 g/liter; 5 μl of each sample was analyzed by agarose gel electrophoresis. Gels were either stained with ethidium bromide and photographed or denatured as described by Matsumoto et al. (10), transferred to nitrocellulose filters, and probed with ^{32}P-labeled RNA made with T7 RNA polymerase (Stratagene) from cDNA clone ge12 containing about 1,800 bases of the 20S RNA sequence (>60% of the total 20S RNA sequence [10]). Hybridizations and washes were carried out under stringent conditions (9).

The protein in each fraction was concentrated by trichloroacetic acid precipitation. One hundred microliters of 100% trichloroacetic acid was added to 400 μl from each fraction, incubated on ice for 60 min, and centrifuged for 15 min in a Beckman Microfuge. Pellets were washed once with 0.5 ml of sterile distilled water and resuspended in 30 μl of sodium dodecyl sulfate-polyacrylamide gel electrophoresis loading buffer (7); 10 μl of each sample was analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Gels were either stained with Coomasie blue or transferred to nitrocellulose filters and probed with Hsp26-specific antibody (17). Antibody detection was with Gamma-Bind G-AP alkaline phosphatase conjugate (GENEX).

Figure 1A shows the results obtained for the *HSP26* (wild-type) strain; 20S RNA sedimented at nearly the same position (slightly faster) as a polypeptide of approximately 29 kDa. This polypeptide must correspond to the 23-kDa protein described previously by Wejksnora and Haber (18), since it is the only low-molecular-weight protein which nearly cosediments with the 20S RNA. The immunoblot

TABLE 1. *S. cerevisiae* strains used in this study

Strain	Characteristics[a]	Source
3234	*MATα his3⁻ leu2⁻ trp1 ura2* 20S⁺ K⁻	Mick Tuite
3235	*MATa his3⁻ leu2⁻ trp1 ura2 HSP26::HIS3* 20S⁺ K⁻	Mick Tuite
2943	*MATa ura3 arg1* L-A-o M-o 20S-o	This work

[a] K⁻, nonkiller; L-A-o, absence of L-A double-stranded RNA; M-o, absence of M double-stranded RNA; 20S-o, absence of 20S RNA; ⁻, doubly mutant alleles.

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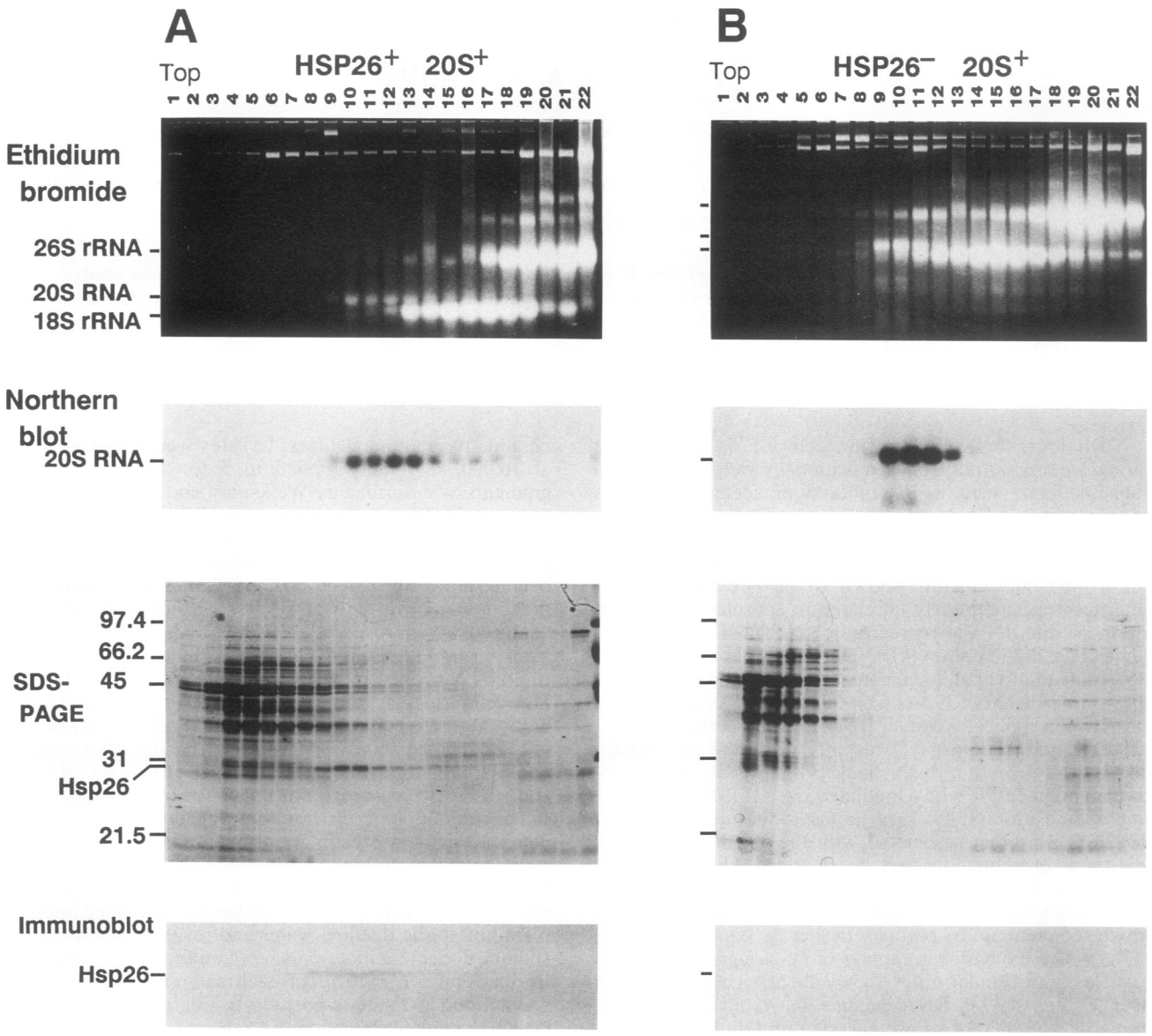

shows definitively that this protein is the host-encoded Hsp26 protein. The synthesis of this polypeptide, however, is apparently not important for 20S RNA production. A strain which has a disruption in the *HSP26* gene still synthesized the 20S RNA at levels comparable to those of the isogenic *HSP26* strain (Fig. 1B). As expected, the Hsp26 protein, which normally would be found sedimenting at about 20S, is not synthesized by this strain.

Not only is 20S RNA synthesis unaffected by the absence of the Hsp26 protein, but it also sediments at essentially the same rate (compare the sedimentation of 20S RNA in Fig. 1A and B with that of the small ribosomal subunits present in each gradient). Furthermore, phenol-extracted (deproteinized) 20S RNA (Fig. 1C) sediments at the same rate as crude 20S RNA samples from *HSP26* (Fig. 1A) or *hsp26* mutant (Fig. 1B) strains, implying that the sedimentation rate is inherent in the RNA itself and is not due to any association with a protein component. Finally, a strain which does not harbor the 20S RNA virus still produces the Hsp26 particles (Fig. 1D). This is in contradiction to the results reported by Wejksnora and Haber (18), who found that the loss of 20S RNA correlated with an inability to synthesize the 23-kDa protein and form particles. Our finding is consistent with the notion that the 23-kDa protein is Hsp26 and is neither encoded nor induced by the virus.

In conclusion, if the 20S RNA does associate with the Hsp26 heat shock protein, it is a loose association and it is not required for viral replication and maintenance. The fact that we see a slight difference in the sedimentation rates of 20S RNA and Hsp26 particles (Fig. 1A) implies that there is no tight complex formed as was previously proposed (18). Our data indicate that the sedimentation rate of naked (phenol-extracted) 20S RNA is indistinguishable from the sedimentation rate of 20S RNA derived from crude extracts. A 2,500-base-pair cDNA (ca. 90% of the viral genome length) has been sequenced, and there is strong potential for

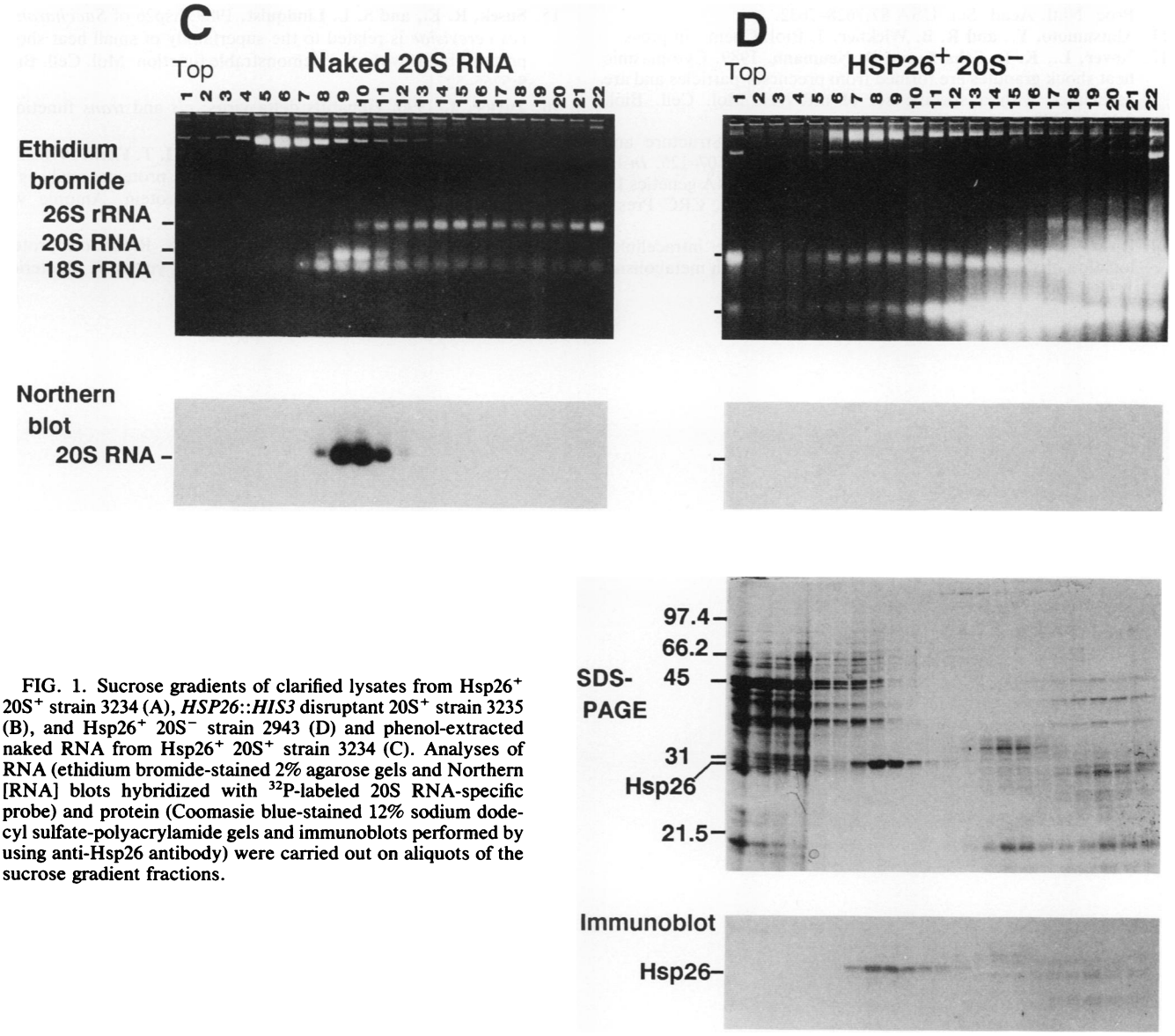

FIG. 1. Sucrose gradients of clarified lysates from Hsp26$^+$ 20S$^+$ strain 3234 (A), *HSP26*::*HIS3* disruptant 20S$^+$ strain 3235 (B), and Hsp26$^+$ 20S$^-$ strain 2943 (D) and phenol-extracted naked RNA from Hsp26$^+$ 20S$^+$ strain 3234 (C). Analyses of RNA (ethidium bromide-stained 2% agarose gels and Northern [RNA] blots hybridized with ^{32}P-labeled 20S RNA-specific probe) and protein (Coomasie blue-stained 12% sodium dodecyl sulfate-polyacrylamide gels and immunoblots performed by using anti-Hsp26 antibody) were carried out on aliquots of the sucrose gradient fractions.

extensive secondary structure (11), which may explain its stability. If 20S RNA is indeed naked (lacking a capsid or envelope) in the cell, it would, in this regard, resemble other circular single-stranded RNA replicons (for reviews, see references 13 and 16).

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REFERENCES

1. **Arrigo, A.-P., J.-L. Darlix, E. W. Khandjian, M. Simon, and P.-F. Spahr.** 1985. Characterization of the prosome from *Drosophila* and its similarities with the cytoplasmic structures formed by the low molecular weight heat shock proteins. EMBO J. **4**:399–406.
2. **Arrigo, A.-P., M. Simon, J.-L. Darlix, and P.-F. Spahr.** 1987. A 20S particle ubiquitous from yeast to human. J. Mol. Evol. **25**:141–150.
3. **Bossier, P., I. T. Fitch, H. Boucherie, and M. F. Tuite.** 1989. Structure and expression of a yeast gene encoding the small heat-shock protein Hsp26. Gene **78**:323–330.
4. **Garvik, B., and J. E. Haber.** 1978. New cytoplasmic genetic element that controls 20S RNA synthesis during sporulation in yeast. J. Bacteriol. **134**:261–269.
5. **Kadowaki, K., and H. O. Halvorson.** 1971. Appearance of a new species of ribonucleic acid during sporulation in *Saccharomyces cerevisiae*. J. Bacteriol. **105**:826–830.
6. **Kadowaki, K., and H. O. Halvorson.** 1971. Isolation and properties of a new species of ribonucleic acid synthesized in sporulating cells of *Saccharomyces cerevisiae*. J. Bacteriol. **105**:831–836.
7. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227**:680–682.
8. **Lindquist, S.** 1986. The heat-shock response. Annu. Rev. Biochem. **55**:1151–1191.
9. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning, a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
10. **Matsumoto, Y., R. Fishel, and R. B. Wickner.** 1990. Circular single-stranded RNA replicon in *Saccharomyces cerevisiae*.

Proc. Natl. Acad. Sci. USA **87**:7628–7632.
11. **Matsumoto, Y., and R. B. Wickner.** J. Biol. Chem., in press.
12. **Nover, L., K.-D. Scharf, and D. Neumann.** 1989. Cytoplasmic heat shock granules are formed from precursor particles and are associated with a specific set of mRNAs. Mol. Cell. Biol. **9**:1298–1308.
13. **Owens, R. A., and R. W. Hammond.** 1988. Structure and function relationships in plant viroid RNAs, p. 107–125. *In* E. Domingo, J. J. Holland, and P. Ahlquist (ed.), RNA genetics II. Retroviruses, viroids, and RNA recombination. CRC Press, Inc., Boca Raton, Fla.
14. **Rossi, J. M., and S. L. Lindquist.** 1989. The intracellular location of yeast heat-shock protein 26 varies with metabolism. J. Cell Biol. **108**:425–439.
15. **Susek, R. E., and S. L. Lindquist.** 1989. hsp26 of *Saccharomyces cerevisiae* is related to the superfamily of small heat shock proteins but is without a demonstrable function. Mol. Cell. Biol. **9**:5265–5271.
16. **Taylor, J.** 1990. Hepatitis delta virus: *cis* and *trans* functions required for replication. Cell **61**:371–373.
17. **Tuite, M. F., N. J. Bentley, P. Bossier, and I. T. Fitch.** 1990. The structure and function of small heat shock proteins: analysis of the *Saccharomyces cerevisiae* Hsp26 protein. Antonie van Leeuwenhoek **58**:147–154.
18. **Wejksnora, P. J., and J. E. Haber.** 1978. Ribonucleoprotein particle appearing during sporulation in yeast. J. Bacteriol. **134**:246–260.